RED DROP AND ROLE OF AUXILIARY PIGMENTS IN PHOTOSYNTHESIS

R. EMERSON AND E. RABINOWITCH [1]

PHOTOSYNTHESIS PROJECT, BOTANY DEPARTMENT, UNIVERSITY OF ILLINOIS

Note: When Robert Emerson was killed in a plane accident on February 4, 1959, much of the experimental material accumulated in his two years work on the action spectrum of photosynthesis in the far-red region, remained unpublished. He was to present these results to the Botanical Congress in Montreal in August, 1959. Instead, the following paper was presented, prepared on the basis of Emerson's earlier talks and laboratory notes, and after consultation with his collaborators, R. V. Chalmers and C. Cederstrand. The theoretical discussion of the results is my own. The presentation of this paper was intended as a memorial to Dr. Emerson; I was urged to publish it to give all those working in the field access to the results of Emerson's last, exciting results. A paper presented by J. Myers (12) at the same meeting provides both confirmation and interesting expansion of the phenomenon which is becoming known as the Emerson effect. In particular, it shows that the so-called chromatic transient, as observed after the replacement of illumination with light at 700 mμ by illumination with a shorter-wave light giving the same steady yield of photosynthesis, has the same action spectrum as the Emerson effect, i.e., suggests a specific role of chlorophyll b.*—E. RABINOWITCH.

INTRODUCTION

From experiments on the action spectrum of photosynthesis (largely by Emerson and co-workers), and of chlorophyll fluorescence (by French, and Duysens) performed up to 1957, an apparently consistent picture emerged which could be summarized as follows.

Photosynthesis is sensitized, in algae of all divisions (and in the higher plants as well), most effectively by light quanta absorbed by chlorophyll a. Light absorbed by other, accessory pigments (chlorophyll b and c, the phycobilins, and the carotenoids) can contribute to photosynthesis with an efficiency which is either lower, or, at best, equal to that of the light absorbed by chlorophyll a. The efficiency of accessory pigments in sensitizing photosynthesis closely parallels their efficiency in sensitizing the fluorescence of chlorophyll a. It is therefore plausible to postulate that the contribution of accessory pigments to photosynthesis is based on transfer of the light energy absorbed by them to chlorophyll a (where it can be utilized to produce chlorophyll fluorescence, or to sensitize photosynthesis). A mechanism by which this transfer is likely to occur has been demonstrated by physicists (Perrin, Vavilov, Förster, and others), and is known as "resonance energy transfer" or "inductive resonance."

The efficiency of the energy transfer to chlorophyll from the several other pigments present in chloroplasts must depend mainly on two factors: the intimacy of their association, which may involve both spatial closeness and chemical attachment, and the overlapping of the fluorescence band of the accessory pigment and the absorption band of chlorophyll a (which is the measure of resonance between the two pigments).

If this picture is accepted, it can still be asked if the energy supply to chlorophyll a is the *main* purpose of the presence of the different accessory pigments in various photosynthetic organisms, or if some of them may have other physiological functions. In the case of the phycobilins, found mainly (but not only) in deep-water red algae, the energy-collecting function appears plausible and may be vital, because of the predominance, deep under the sea, of green light not effectively absorbed by chlorophyll. This fact, and the high efficiency of energy transfer from the phycobilins to chlorophyll (80–95 %), observed in measurements of the action spectrum of chlorophyll fluorescence, supports the assumption that energy supply is in fact the main raison d'etre of red (and blue) pigments in Rhodophyceae. The case of fucoxanthol appears similar; this pigment, too, contributes significantly to the absorption of submarine light by the Phaeophyceae and Bacillariophyceae, and experiments show a high efficiency of energy transfer from it to chlorophyll a. Other carotenoids, particularly those found in green plants, contribute very little to the total absorption of sunlight, and the efficiency of energy transfer from them to chlorophyll a is relatively low (20–50 %). It seems likely that the main physiological function of these pigments is different; the fact that they contribute excitation energy to chlorophyll a may well be merely a physically unavoidable, but physiologically insignificant phenomenon.

The above-mentioned picture had one dark spot. Blinks, Haxo, and Yocum (9) had concluded, from measurements of the action spectra of photosynthesis in various species of red algae, that the photosynthetic efficiency of the light quanta absorbed by the phycobilins is generally *higher*; often much higher, than that of the light quanta absorbed by chlorophyll a. This suggested direct sensitization of photosynthesis by the red pigment, without the intermediary of chlorophyll a—in contradiction to the above-described picture. However, Duysens (3) found that a similar phenomenon can be observed also in the excitation of the fluorescence of chlorophyll a in these algae; it, too, is excited *more efficiently* by light quanta absorbed by the phycobilins than by those absorbed by chlorophyll a itself. The hypothesis that the accessory pigments

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* Perhaps, it should be called the "second Emerson Effect", the first being the carbon dioxide burst at the beginning of the illumination period.

contribute to photosynthesis by transferring their excitation energy to chlorophyll a thus seemed to be valid in this case, too; it was, however, paradoxical that light absorption by chlorophyll itself appeared to produce excited chlorophyll molecules less apt to sensitize photosynthesis, or to emit fluorescence, than the excited chlorophyll molecules obtained by energy transfer from the accessory pigments.

I. Red Drop. Emerson decided to investigate this paradoxical situation in 1958. The detailed investigation of the action spectrum of photosynthesis in Porphyridium, made in collaboration with M. Brody (1), changed the experimental picture substantially. The relative inefficiency of light absorbed by chlorophyll a in red algae proved to be limited to the part of the absorption spectrum above 650 mμ; while at 644 mμ (where the light absorption is divided about equally between chlorophyll and phycocyanin), the absorbed quanta were found to be generally more effective than the 546 mμ quanta, absorbed entirely by the phycobilins. In the extreme case (that of algae adapted to green light, which is absorbed predominantly by phycoerythrin), the two efficiencies were equal (cf. fig 1). This suggests a situation similar to that found in other divisions of algae, where the efficiency of the accessory pigments can equal, but not exceed, that of chlorophyll.

It remains to be seen whether a correction similar to that brought by M. Brody's measurements to the action spectrum of *photosynthesis* of red algae, must be applied also to the above-mentioned conclusions of Duysens et al, concerning the action spectrum of chlorophyll a *fluorescence* in red algae; in other words, whether or not the quantum yield of directly excited chlorophyll fluorescence also declines in these cells only above 650 mμ.

The puzzle of the action spectrum of photosynthesis in red algae was shifted by M. Brody's measurements

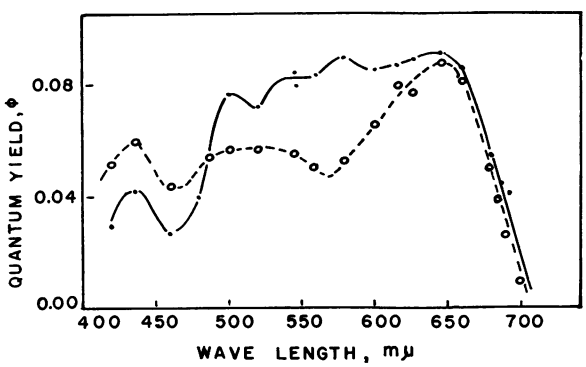


Fig. 1. Action spectra of photosynthesis in Porphyridium pre-illuminated with green light (solid line) and blue light (dashed line). In the first case, the phycobilins are about as efficient in sensitizing photosynthesis as chlorophyll; in the second case they are only about half as effective. There is no significant difference in the pigment content of the two samples. [After M. Brody and R. Emerson (1)].

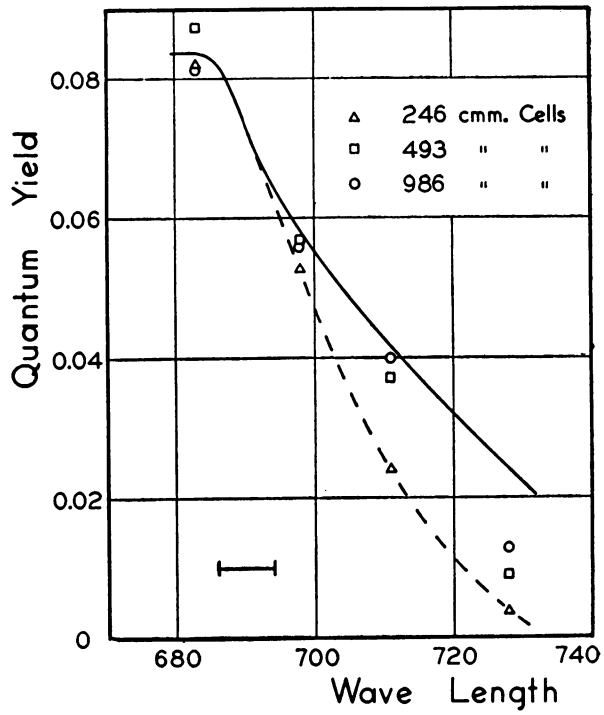


Fig. 2. Red end of the action spectrum of Chlorella. Solid curve is extrapolation of results to complete absorption. Width of spectral bands used is indicated. [After R. Emerson and C. Lewis (6)].

from the alleged low effectiveness of chlorophyll a *as such*, to the low efficiency of light absorbed by chlorophyll a > 650 mμ. It seemed significant that this is the region where chlorophyll a becomes the *only* absorbing pigment; one had to recall the observation, made by Emerson and Lewis (6) in 1943, that in Chlorella, too, the quantum yield of photosynthesis declines in the extreme red part of the chlorophyll absorption band, > 685 mμ—the region where the absorption by chlorophyll b is likely to be negligible, and practically all absorption must be due to chlorophyll a, (cf. fig 2). Emerson therefore conceived a working hypothesis, which seemingly ran contrary to the above-outlined generally accepted concept. He suggested that in order for photosynthesis to proceed with maximum efficiency, energy quanta must be contributed by *two pigments*: by chlorophyll a, *and* by one of the auxiliary pigments—chlorophyll b in green algae, a phycobilin in red algae, etc.

Although the red drop in the quantum yield of photosynthesis of green algae above 680 mμ was rather conclusively established by the 1943 study, the rapidly declining absorptive capacity of the cells in this region and their capacity for selective scattering of light on the long-wave side of the absorption peak (discovered by Latimer (10), cf. fig 3) permitted some doubt as to the reality of the drop; it could conceivably have been caused by overestimation of the number of quanta actually absorbed by chlorophyll in this region. A

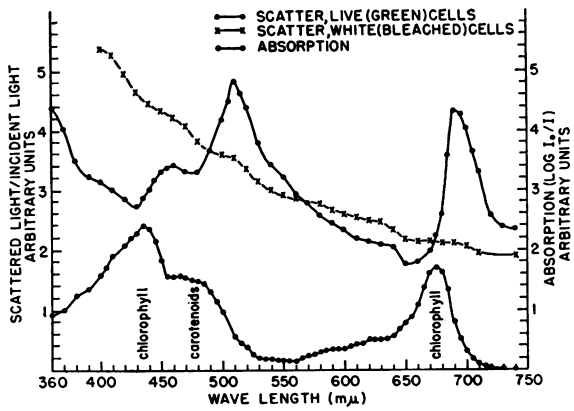
more precise study of the red drop was therefore undertaken by Emerson and Chalmers. Preliminary results have been presented in two communications to the National Academy of Sciences (5). Some speculations on the implications of the findings were discussed in a talk (4) before the Phycological Society. The main body of results remained unpublished at the time of Emerson's death.

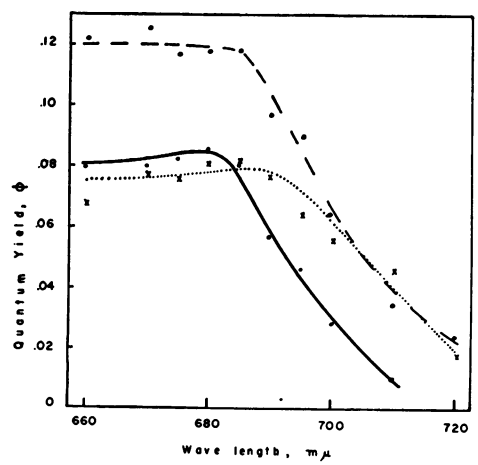
II. Enhancing Effect of Auxiliary Light. The far-red quantum drop made many people think that perhaps in this part of the spectrum, energy quanta may be too small to sustain photosynthesis, or at least to do so with a good yield. This seemed an attractive explanation, despite the fact that spectroscopic theory suggests that within a given electronic absorption band, the electronically excited molecules, after thermal equilibration with the medium (which is likely to be completed, in a condensed medium, before the photochemical reaction takes place), must have the same vibrational energy distribution, and thus also identical reactivity, whatever the specific size of the absorbed quantum.

If insufficient vibrational energy of the electronically excited chlorophyll molecules had something to do with the red drop, one could expect the quantum yield in this spectral region to improve with increasing temperature. The first experiments of Emerson et al (5) were therefore concerned with the effect of temperature on the quantum yield in the far red. A temperature effect was in fact found, but it had the opposite sign from that expected; the far red light was used more effectively at 5° C than at 20° C! (fig 4).

This finding, which in any case did not support the suggested interpretation of the red drop, was not further pursued, since another one, obtained at about the same time, appeared more exciting, supporting, as it did, the interpretation of the drop as indicative of the importance, for photosynthesis, of quanta absorbed by accessory pigments. This was the finding (figs 4, 5) that the quantum yield of photosynthesis in far red light can be substantially improved by auxiliary illumination with light of shorter wave length. The average quantum yield obtained in two superimposed beams proved to be substantially higher than the average of the quantum yields obtained by using the two light beams separately. Since in the auxiliary light per se, the yield was the usual one (8–12 quanta per molecule of O_2), while in the far red beam per se it was substandard (20–50 quanta per molecule of O_2), it was natural to ascribe the effect of the mixing of two lights to the improvement of the photochemical effectiveness of the quanta of far red light by the auxiliary light. (The average quantum requirement of two combined beams never was below the familiar minimum of 8 to 12 quanta.

The next question was: what was important, the *size* of the quanta of the auxiliary light, or the *pigment* that absorbed them? This required a determination of the action spectrum of the auxiliary light, a task that proved extremely laborious and time-consuming. The method used was to combine far red illumination





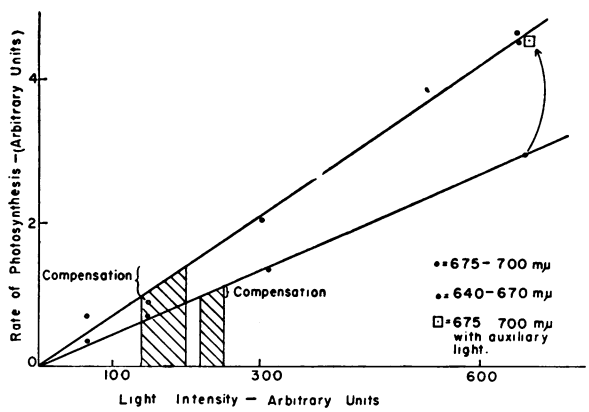


Fig. 3. Selective scattering of light by Chlorella cell suspension. [After P. Latimer and E. Rabinowitch (10)].

Fig. 4. Red drop of quantum yield of photosynthesis in Chlorella. Solid line, 5° C; dashed line 20° C; dotted line, 20° C with supplementary light of shorter wavelength. [After R. Emerson, R. V. Chalmers, and C. Cederstrand (5)].

Fig. 5. Proportionality of the rate of photosynthesis to light intensity in far-red and auxiliary light. [After R. Emerson, R. V. Chalmers, and C. Cederstrand (5)].

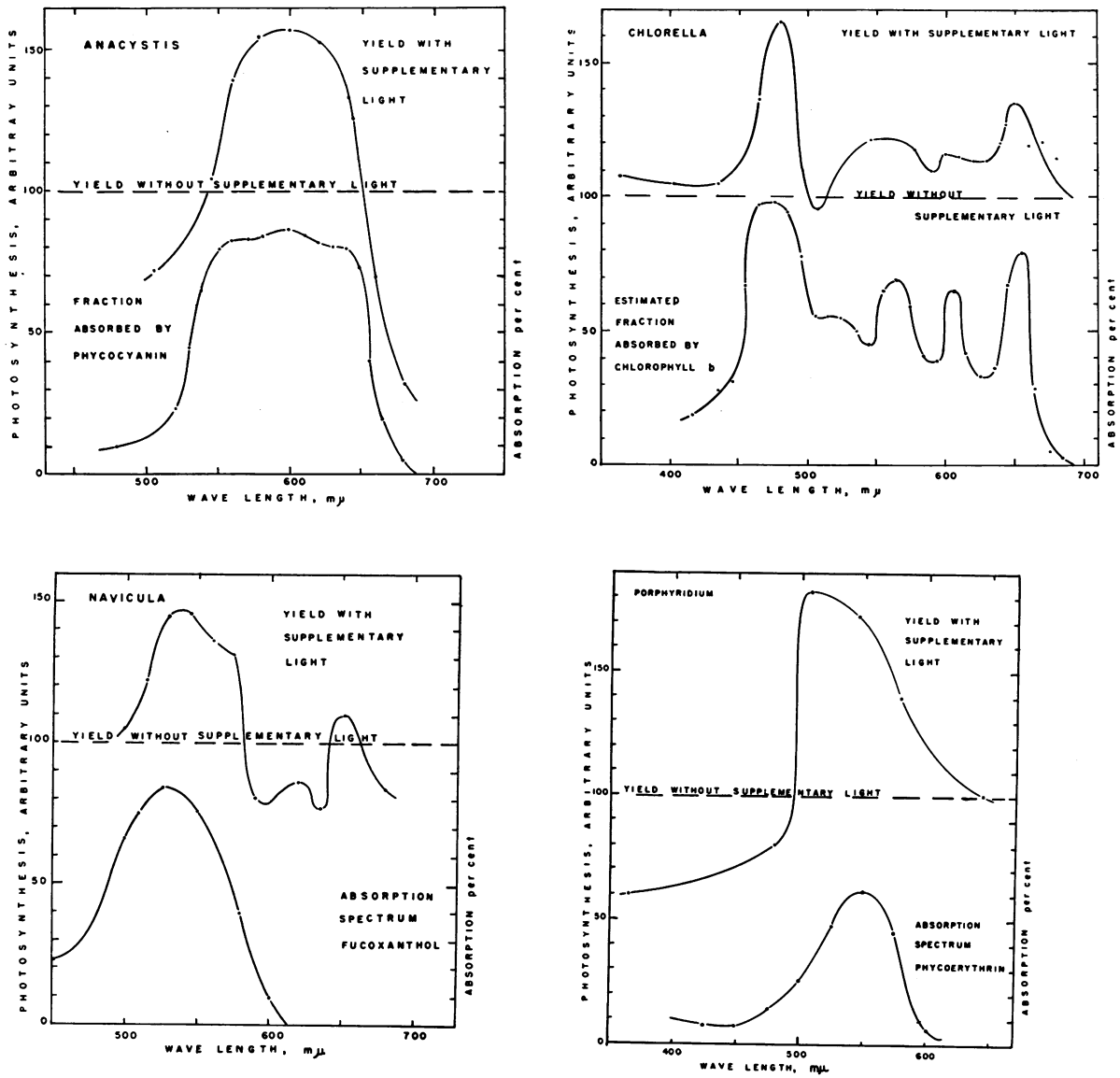


FIG. 6. Effect of monochromatic supplementary light on the yield of photosynthesis in far red light for the blue-green alga Anacystis, compared to the fraction of light absorbed by phycocyanin. [After R. Emerson, R. V. Chalmers, and C. Cederstrand, unpubl.].

FIG. 7. Effect of monochromatic supplementary light on the yield of photosynthesis in far-red light for the green alga Chlorella, compared to the fraction of light absorbed in chlorophyll b. [after R. Emerson, R. V. Chalmers, and C. Cederstrand, unpubl.].

FIG. 8. Effect of monochromatic supplementary light on the yield of photosynthesis in far-red light for the diatom Navicula, compared to the absorption spectrum of fucoxanthol. [after R. Emerson, R. V. Chalmers, and C. Cederstrand, unpubl.].

FIG. 9. Effect of monochromatic supplementary light on the yield of photosynthesis for the red alga Porphyridium, compared to the absorption spectrum of phycoerythrin. [after R. Emerson, R. V. Chalmers, and C. Cederstrand, unpubl.].

of certain constant intensity with the illumination by auxiliary light of an intensity giving, by itself, approximately the same amount of photosynthesis. The curve showing the yield of combined illumination, minus the yield caused by the auxiliary light alone, was plotted as function of wave length of the auxiliary light, and compared with the curve showing the relative proportion of light absorbed, at the same wavelengths, by the auxiliary pigment (or simply with the absolute absorption curve of this pigment).

Figures 6 to 9 show this comparison for algae of four divisions, containing phycocyanin, chlorophyll b, fucoxanthol, and phycoerythrin as main auxiliary pigment. In every case, the action spectrum of supplementary light is strikingly similar to the (relative or absolute) absorption spectrum of the auxiliary pigment.

The chosen method of comparison implied that the stimulating effect of auxiliary light on the far-red yield is proportional to (or at least, rises monotonously with) the intensity of the auxiliary light. This was checked by experiments with constant intensity of far-red, and varying intensity of auxiliary light. It clearly shows that the effect of auxiliary light is by no means a catalytic one [as suggested by Warburg (14) for the special case of blue light added to light of greater wave lengths], but rises with its intensity until it begins to show saturation, in the same intensity region where photosynthesis becomes light-saturated.

Considering the uncertainties involved in the calculation, the parallelism of the curve pairs in figures 6 to 9 appears striking. It justifies the conclusion that the enhancing effect of light of a certain wavelength on the yield in the far red parallels the proportion of light absorbed, at this wavelength, by the main auxiliary pigment—chlorophyll b in Chlorella, phycocyanin in Anacystis, fucoxanthol in Navicula, and phycoerythrin in Porphyridium. It seems that the far-red quanta, absorbed by chlorophyll a, are most effectively supplemented by quanta absorbed by an auxiliary pigment, and not by other quanta absorbed by chlorophyll a itself.

Since in an average higher plant a broad band of red and orange light, which is absorbed to about ¼ by chlorophyll b and about ¾ by chlorophyll a, can produce photosynthesis with full efficiency, it seems that quanta absorbed by chlorophyll a at the shorter wavelengths do not need supplementation by quanta absorbed by other pigments.

One matter that needs further attention is the appearance on figures 6 to 9, of *negative* effects. If confirmed by further experiments, they may mean that under certain conditions, combinations of far-red light with light of shorter wave lengths may decrease rather than increase the total yield (even though the experiments were carried out at light intensities far below saturation). In this case, it may be that the effect is due to decline in the efficiency of the auxiliary short-wave light to below the usual value of 8 to 10 quanta per O_2 molecule, rather than to a further decline in the efficiency of the far-red light. It is very likely that the need for balancing the excitation of the several pigments present in chloroplasts manifests itself in various ways, and not only in the inefficiency of far-red light absorbed only by chlorophyll a.

III. Experiments on Xanthophyceae (Heterokontae). According to data in the literature (13) Xanthophyceae contain no chlorophyll b. If Emerson's hypothesis is correct, they should show no effect of supplementary light on the yield in far-red light. This was in fact confirmed by experiments with Polyedriella. However, measurements of action spectra showed that this difference was due to the extension of high quantum yields out to 690 mμ, and *not* (as was expected) to the drop of the yield > 680 mμ being insensitive to auxiliary light! Since these algae were grown in a medium containing organic nutrients, it was suspected that the presence of such nutrients may permit full photosynthetic rate at 690 mμ, without the aid of supplementary light. Preliminary experiments by R. Govindjee suggested that if Polyedriella is precipitated from the organic and transferred into the inorganic medium, and the transfer is repeated several times, the red drop does appear after about five transfers. This suggests that Polyedriella grown in organic medium may contain a substrate (or a catalyst) which permits the cell to utilize light at 680 to 690 mμ with full efficiency, without the help of light of shorter wavelengths.

To test on Polyedriella the theory of chlorophyll b absorption as source of the auxiliary light effect on the yield in the far red, one would have to make the experiment on Polyedriella cultures repeatedly transferred into an inorganic medium; and this experiment has not yet been carried out.

IV. Effects of Organic Nutrition on Red Drop in Chlorella and Porphyridium. The observations on Polyedriella raised the question whether the red drop in other algae also could be affected by organic nutrition. Experiments with Chlorella grown in glucose solution showed no change, but algae grown in the presence of beef broth or earth extract did show a shift of the red drop to long wave lengths, (fig 10).

Because of Myers' findings in experiments on algal growth, it was suspected that vitamin K may be a possible active ingredient of broth or earth extract; experiments with the addition of this (and other) pure organic micronutrients are planned.

Even considerable alterations in the composition of the *inorganic* growth medium had no effect on the red drop in Chlorella.

V. Effect of Time Interval Between Absorption of Far-red Light and Supplementary Light. In the experiments described above, the experimental arrangement was that shown in figure 11. The far-red light, isolated by glass filters, entered the manometer vessel from above, while the auxiliary light came from below, usually through a monochromator (except in blue-violet light, where filters had to be

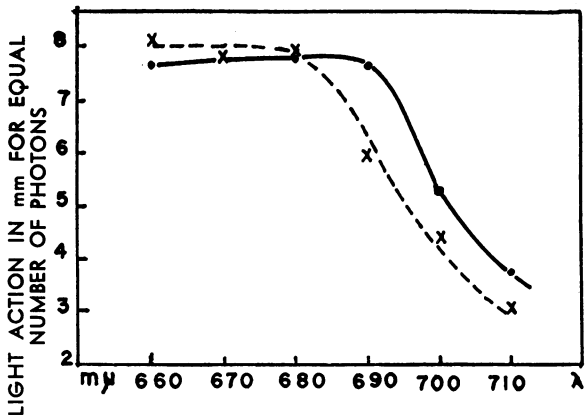


FIG. 10. Effect of earth extract on the red drop in Chlorella. [after R. Emerson and R. Govindjee, unpubl.].

used). In the majority of the experiments, both beams were practically completely absorbed. In a preliminary experiment, the auxiliary beam was sent from below and the far-red light from the side, and the enhancing effect was found to be considerably smaller. Since in the second arrangement, a cell needs less time to get from the bottom layer of the vessel, (where it has the greatest chance to absorb auxiliary light) into the layer where its chance to absorb far-red light is highest, this observation raised the possibility that a certain *time interval* between the two absorption acts may favor the effect. This suggests experiments in which the two beams would be alternated at variable time intervals.

VI. INTERPRETATION OF RED DROP. Emerson's conclusion that photosynthesis required (or, at least, is enhanced by) energy absorption in an auxiliary pigment, permits of several hypothetical interpretations. The simplest one is to postulate that two (or more) different primary photochemical processes are involved in photosynthesis, and are preferentially sensitized by the several pigments. For example, one could imagine that photosynthesis requires both *photoreduction* (of CO_2 or of a substrate derived from it, such as a carboxylic acid), and *photoxidation* (of water or a substrate derived from it). One could describe essentially the same concept by saying that each hydrogen atom (or electron) must be photoactivated *twice* on its path from the ultimate donor, water, to the ultimate acceptor, carbon dioxide, and that each of these two steps is preferentially sensitized by one or the other of the pigments. To use a concrete example, one could imagine a mechanism of the following type:

$$\text{I.}\quad \text{Chl a} + H_2O \xrightarrow{h\nu} O_2 + \text{r Chl a}$$

$$\text{II.}\quad \text{Chl b} + CO_2 \xrightarrow{h\nu} \left\{ CH_2O \right\} + \text{o Chl b}$$

$$\text{III.}\quad \text{o Chl b} + \text{r Chl a} \rightarrow \text{Chl b} + \text{Chl a}$$

—chlorophyll a serving preferentially as photoxidant and chlorophyll b (or another auxiliary pigment) preferentially as photoreductant (or vice versa), and a dark reaction, III, between the reduced and the oxidized pigment restoring the original system. (I say "preferentially" because in the steady state, reactions I and II must proceed at the same rate; if only Chl b could sensitize reaction II, the maximum yield could be obtained only in light absorbed to one-half by chlorophyll b, and one-half by chlorophyll a, and not in a broad red-orange band absorbed to only one-fourth by the b component.)

The above interpretation of Emerson's findings is made unlikely, if not impossible, by the evidence, derived from fluorescence experiments, that of the quanta absorbed by chlorophyll b (as well as by phycobilins, or fucoxanthol), a very large fraction is transferred to chlorophyll a by resonance. This evidence is not as precise as could be desired, and the relevant experiments need to be repeated with improved techniques; but on the whole, they seem convincing.

It would be very difficult to explain the approximate equality of the quantum yields of fluorescence of chlorophyll a when excited by light absorbed by this pigment itself and when excited by light absorbed by chlorophyll b (or fucoxanthol) without assuming that most of the quanta absorbed by these auxiliary pigments are transferred to chlorophyll a. (True, the actual fluorescence yield is only, $< 3\,\%$, but it is a reliable index of what happens to the totality of the absorbed quanta).

An alternative interpretation of Emerson's results is to begin with the fact that most of the quanta absorbed by the auxiliary pigments are transferred to chlorophyll a, and thus cannot produce directly any specific photochemical effects. Instead, chlorophyll

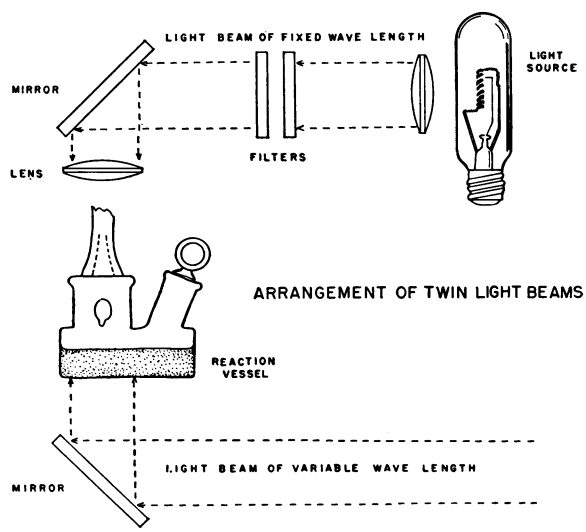
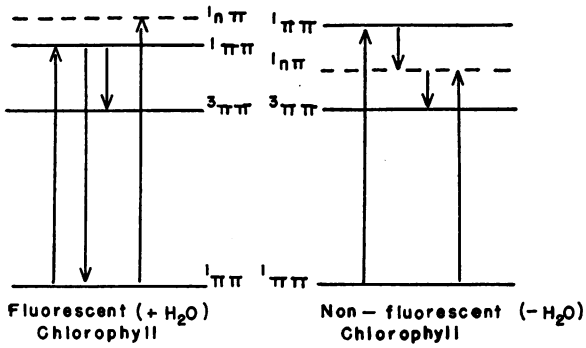


FIG. 11. Arrangement of two light beams in experiments with antiparallel beams. [after R. Emerson, unpubl.].



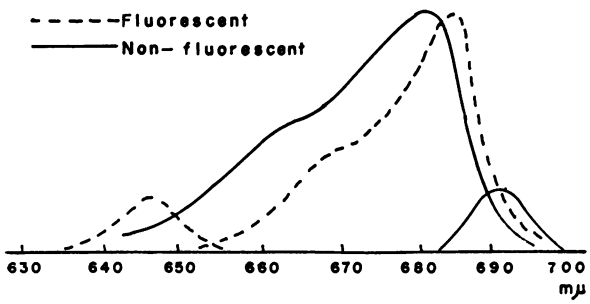


FIG. 12. Order of energy levels in fluorescent and non-fluorescent forms of chlorophyll. (Lowest $^1\pi\pi$ level, S, higher $^1\pi\pi$ level, S; $^3\pi\pi$ level is T.)

FIG. 13. Possible shapes of the absorption curves of chlorophyll in the fluorescent state (dashed line) and in the non-fluorescent state (solid line). In the first case, the n π band lies on the short-wave side of the main $\pi\pi$ band; in the second case, it lies on its long-wave side, providing a possible explanation of the red drop.

a, carrying the transferred quanta, must be postulated to bring about preferentially a primary photochemical process different from the one caused (preferentially) by the long-wave quanta absorbed by chlorophyll a itself. To make this plausible, one could postulate, for example, the existence of two types of chlorophyll a complexes in the cell, e.g., such associated with a reductant and such associated with an oxidant, and assume that one of these two types is closer to the auxiliary pigment than the other.

Somewhat awkward is the necessity to postulate equal fluorescence yield of both types. Livingston (11) has shown how different is, for example, the fluorescence of chlorophyll associated with polar molecules (water, alcohol, amines) from that of chlorophyll surrounded exclusively by non-polar lipoid molecules. The fluorescence efficiency is generally determined by competition between the radiative transfer from the fluorescent (excited singlet) state to the ground singlet state, and the non-radiative transfer to the metastable triplet state (fig 12). It has been often postulated that the photochemical process is initiated by long-lived molecules in the triplet state; this explains why it often does not compete with fluorescence, i.e., why the substrate of the photochemical reaction often does not quench fluorescence. (This does not mean that photochemical processes *cannot* be initiated by the S*-state—in which case the substrate will quench the fluorescence).

The result of the competition between the processes S* → S and S → T in figure 12 can be strongly affected by the presence of a third, (so-called n π) excited state. If this state is located *between* the S*-and the T-state, its presence catalyzes the conversion to triplet, and thus reduces or altogether quenches fluorescence. If the n π-state is located *above* the S*-state, it has no such effect. This is the presently accepted explanation of the enhancing effect of polar molecules on the fluorescence of chlorophyll: the proximity of polar molecules shifts the n π-state upwards and the S*-state downwards, thus favoring fluorescence. In the hypothesis we are now considering, the two forms of chlorophyll complexes must both belong to the same, fluorescent type.

Franck (7) suggested a different interpretation. In it, the two kinds of chlorophyll involved in photosynthesis are a non-fluorescent form, in which the excited molecules in the S*-state are instantaneously converted, via the n π-state, into metastable molecules T; and a fluorescent kind, which permits the S*-state to survive long enough either to fluoresce, or to migrate, by resonance, through a sequence of chlorophyll molecules, until transferred to a molecule already in the T-state, raising it into an *excited triplet* state, T*. The latter has an energy content so high as to permit direct sensitization of an electron (or hydrogen) transfer from H_2O to RCOOH (or their equivalents on the energy scale).

Absorption on the long-wave side of the main absorption peak, > 690 mμ, is suggested, in Franck's theory, to be due predominantly to n π transitions in the non-fluorescent state (fig 13) it therefore produces predominantly T-molecules, which *alone* cannot bring about photosynthesis. Absorption in the main band, 650 to 690 mμ, on the other hand, excites both

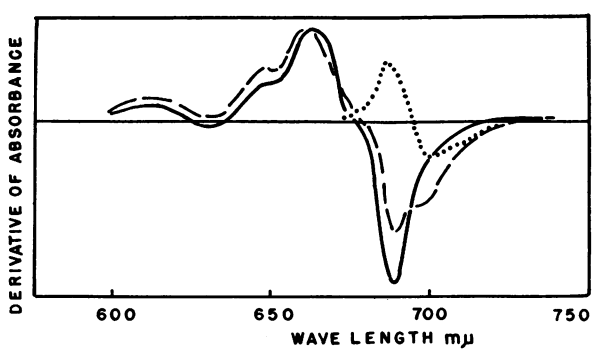


FIG. 14. Differential spectra of young and old cultures of Euglena suggesting the existence in vivo of a chlorophyll form with absorption peak at 695 mμ [after C. S. French (8)].

types of chlorophyll. Depending on their ratio, it can either produce about equal numbers of S* and T-molecules, and thus ultimately, a large number of T*-molecules and a high yield of photosynthesis (as in Chlorella), or predominantly T-molecules, and thus a low yield of chlorophyll fluorescence and photosynthesis (as in Porphyridium). Finally, (developing Franck's interpretation so as to fit M. Brody's findings), one can suggest that absorption at 640 to 650 mμ could excite predominantly the fluorescent species (in which the absorption on the short-wave side of the main peak is reinforced by the n π-transition!), and thus give, in all cells, a high yield of fluorescence and of photosynthesis.

It is to be noted that under no condition is there a danger of *shortage* of molecules in the T-state, since even in the extreme case of exclusive excitation of fluorescent molecules, the transition S* → T is the most likely fate of each S*-molecule, *unless* enough T-molecules have accumulated for the transition S* + T → S + T* to become equally likely.

Another note: as Franck pointed out, similar kinetic relationships can be derived also by considering, instead of a double excitation leading to the T*-state, two separate primary photochemical processes, one sensitized by molecules in the S*-state and one sensitized by molecules in the T-state, with the full quantum yield being only possible if the rates of the two processes are equal.

The assumption that there are (at least) two different kinds of chlorophyll in the chloroplasts imposes itself quite independently from Franck's ingenious hypothesis. The width of the red band in vivo alone suggests it (Duysens, personal communication). The analysis of the shape of the absorption band by French (8) provides quantitative evidence that the red band has a complex nature, and that its composition varies from species to species. One minor band, found in the absorption spectrum of some cells by French, has a peak at about 695 mμ, and could thus qualify as the band responsible for the drop in quantum yield of photosynthesis in Chlorella > 685 mμ (fig 14); it may be the n π band of the non-fluorescent chlorophyll component postulated by Franck.

However, French also noted (in Euglena cells) a *fluorescence* band at 705 mμ, which he associated

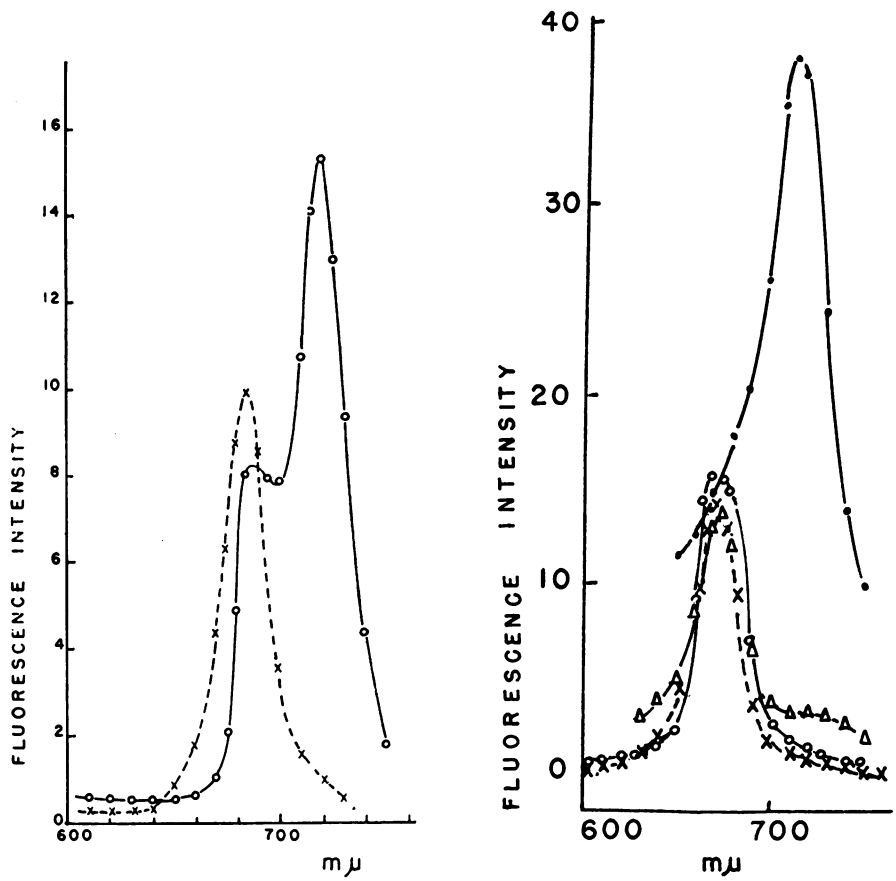


FIG. 15. Fluorescence spectrum of Chlorella at room temperature (dashed line) and at —193° C. (solid line). [after S. S. Brody (2)].

FIG. 16. Fluorescence spectra of chlorophyll a solutions in ethanol. Dots: concentrated solution at —193° C; triangles, same solution at room temperature; circles, dilute solution at —193° C; crosses, same solution at room temperature. [after S. S. Brody (2)].

with the 695 mμ absorption band. S. Brody (2) found a fluorescence band in the same position in low-temperature (liquid air) fluorescence spectrums of Chlorella (fig 15), and of concentrated (but not of diluted!) chlorophyll solutions (fig 16), and attributed it to dimeric (or higher polymeric) chlorophyll molecules. There is no doubt that chlorophyll, similarly to other dyes, does dimerize in concentrated solutions. From concentration quenching data, Weber (personal communication) calculated a dimerization constant of 4.5 for chlorophyll a in ether, which implies about 3 % dimerization at 10^{-2} m/l. The proportion of dimers may increase considerably at low temperature, and it is not implausible that dimeric molecules, non-fluorescent at room temperature, may become fluorescent at −190° C. To what extent dimerization can be expected to occur in chlorophyll in vivo, which is probably adsorbed on protein as a monomolecular layer, is uncertain. Brody suggested that the red drop in the action spectrum of photosynthesis is caused by dimers (or higher polymers) with an absorption band at 695 mμ; and that the much earlier drop in red algae could be explained by the same dimers being present in a much higher proportion.

Whatever specific hypothesis would be supported by future studies, it is highly plausible that the red drop is associated with the existence of several forms of chlorophyll a in vivo, and with the incapacity of one of these forms (or, to be more cautious, of one absorption band of this form) to sensitize photosynthesis with full maximum quantum yield; the enhancing effect of light absorbed by other pigments is most probably due to the preferential transfer of excitation energy from these pigments to another more active form of chlorophyll a.

Summary

The enhancement of the quantum yield of photosynthesis in far red light (>680 mμ) by auxiliary light of shorter wavelengths is measured as a function of the wavelength of auxiliary light, for green (Chlorella), brown (Navicula), red (Porphyridium), and blue-green algae (Anacystis). The resulting "action spectra of the Emerson effect" show sharp peaks in the regions where the contribution of auxiliary pigments (chlorophyll b in Chlorella, chlorophyll c, and fucoxanthol in Navicula, phycoerythrin in Porphyridium, phycocyanin in Anacystis) to total light absorption is highest. This appears offhand as evidence that excitation of chlorophyll a alone is insufficient to produce photosynthesis (at least, not with a high yield), and simultaneous excitation of one of the auxiliary pigments is needed. However, the high yield of chlorophyll fluorescence in vivo sensitized by auxiliary pigments contradicts this interpretation, since it suggests highly efficient transfer of excitation energy from auxiliary pigments to chlorophyll a. A solution can be sought in the assumption of (at least) two different forms of chlorophyll a, which need to be excited to produce photosynthesis. Only one is excited by far red light, the other can be excited either directly, e.g., by near red light, or indirectly, by energy transfer from the auxiliary pigments.

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